Review

COVID-19: A state of art on immunological responses, mutations, and treatment modalities in riposte

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ABSTRACT

Over the last few years, the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) unleashed a global public health catastrophe that had a substantial influence on human physical and mental health, the global economy, and socio-political dynamics. SARS-CoV-2 is a respiratory pathogen and the cause of on-going COVID-19 pandemic, which testified how unprepared humans are for pandemics. Scientists and policymakers continue to face challenges in developing ideal therapeutic agents and vaccines, while at the same time deciphering the pathology and immunology of SARS-CoV-2. Challenges in the early part of the pandemic included the rapid development of diagnostic assays, vaccines, and therapeutic agents. The on-going transmission of COVID-19 is coupled with the emergence of viral variants that differ in their trans-mission efficiency, virulence, and vaccine susceptibility, thus complicating the spread of the pandemic. Our understanding of how the human immune system responds to these viruses as well as the patient groups (such as the elderly and immunocompromised individuals) who are often more susceptible to serious ill-ness have both been aided by this epidemic.

COVID-19 causes different symptoms to occur at different stages of infection, making it difficult to de-termine distinct treatment regimens employed for the various clinical phases of the disease. Unsurprisingly, determining the efficacy of currently available medications and developing novel therapeutic strategies have been a process of trial and error. The global scientific community collaborated to research and develop vaccines at a neck-breaking speed. This review summarises the overall picture of the COVID-19 pandemic, different mutations in SARS-CoV-2, immune response, and the treatment modalities against SARS-CoV-2.
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Contents

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Introduction

COVID-19 (novel COrona VIrus Disease-2019) is a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). On March 11, 2020, the World Health Organization (WHO) declared the COVID-19 outbreak a pandemic. The WHO recorded 4.5 million COVID-19 deaths and over 217 million persons infected by September 2021, making it the biggest SARS coronavirus outbreak ever [1]. Coronaviruses usually cause mild respiratory tract infections in humans, such as the common cold. However, coronaviruses have caused two outbreaks in the past, severe acute respiratory syndrome (SARS) in 2003 and the Middle East Respiratory Syndrome (MERS) in 2012 [2].

The first case of COVID-19 was officially reported in December 2019 and was caused by a novel beta-coronavirus [3]. The outbreak was traced to a seafood market in Wuhan, and according to an initial study 49 out of 99 patients had an exposure history to the Huanan seafood market [4]. Initially, the virus was designated as WH-Human 1 coronavirus; however, was subsequently renamed SARS-CoV-2, and the disease was named COVID-19 on February 11, 2020 [3]. Soon after the first recorded regional outbreak in Wuhan, the disease spread worldwide causing the global pandemic in just few months.

For the understanding of the dynamics of any outbreak, the basic reproductive number (R0) is used as a key epidemiological parameter. The R0 value is important to calculate the percentage of the population that needs to be immunised to achieve herd immunity, using the formula 1–1/R0. In the initial phases of the pandemic, the basic reproductive number (R0) for SARS-CoV-2 ranged from 2.2 to 2.7 and a doubling time of 6–7 days [5]. This R0 value is somewhat comparable with that of the Spanish influenza outbreak of 1918, with an R0 range from 2 to 4 [6]. Due to the lack of public awareness and the population moving across the country for the Chinese Spring Festival, it is estimated that the median R0 was as high as 5.7 (95% CI 3.8–8.9) during the initial phase of the outbreak in densely populated Wuhan [5]. Given an R0 of 2.7, at least 63% of the population needs to be vaccinated to achieve herd immunity [7]. These values are not very reliable as the R0 for COVID-19 is not exact and hard to estimate due to the fluctuating nature of this pandemic.

The other fluctuating figure for COVID-19 was from the onset of symptoms to hospitalisation, which was a median estimated as 5.5 days (95% CI 4.6 – 6.6 days) before January 18, 2020. However, due to increased public awareness of the disease this duration was significantly shortened to 1.5 days (95% CI 1.2 – 1.9 days) [5]. The time from admission to discharge was 11.5 days (95% CI 8.0 – 17.3 days), and from admission to death was 11.2 days (95% CI 8.7 – 14.9 days). However overall time from the onset of symptoms to death was 16.1 days (95% CI 13.1 – 20.2 days) [5]. The median incubation period for COVID-19 is four days (interquartile range, 2 – 7); however, [8,9] in some cases, an extended incubation period of up to 24 days was also reported [10]. Due to the long and infectious nature of the incubation period, quarantine and social distancing to avoid any contact was needed to slow down the incidence rates of COVID-19 [11]. Besides these varying factors, COVID-19 associated fatality rate also varies in different countries, which made it difficult to estimate case fatality ratio [12]. Although the mortality rate was lower than SARS (9.14%) and MERS (34.4%) [10], the number of deaths due to COVID-19 has significantly surpassed SARS and MERS, which is due to the highly contagious nature and a much wider spread of this infection. The purpose of this review is to help readers obtain a better knowledge and understanding of how COVID-19 affected the world, how it affected immune systems of the people, the changes it caused, and how to combat the

pandemic. Hopefully, this will help us better prepare for any potential epidemic in the future.

Study selection

For this study, we searched and reviewed published work on COVID-19 on the commonly used search engines such as PubMed, ScienceDirect and Scopus. The keywords searched for where COVID-19, SARS-CoV-2, immune system to cover the topics related to the immune response. Additionally, terms such as COVID-19, SARS-CoV-2 and vaccine were used to find COVID-19 vaccine related information. The R0 topic was specifically searched into with aid of textbooks. Reports from other sources such as World Health Organization (WHO), Centers for Disease Control and Prevention (CDC) and from other authentic sources have also been exhaustively searched and included in this study. The search period of the information lasted from April 2020 until July 2022. The results were narratively reviewed, grouped, and presented in this review.

Clinical diagnosis, signs, and symptoms

Clinical diagnosis and laboratory testing are the important parameters associated with any pandemics. Due to the lack of awareness and abrupt spread, clinical diagnostics for SARS-CoV-2 remained an initial challenge with COVID-19 pandemic. Furthermore, it was challenging to distinguish COVID-19 from other viral respiratory infections due to the lack of specific clinical features. Diagnostic challenges also remain associated with different phases of the disease manifestations, including asymptomatic, pre-symptomatic, and atypical presentations. Right from the initial stages of the outbreak, two diagnostic methods, including reverse-transcription polymerase chain reaction (RT-PCR) (COVID-19 nucleic acid detection kit) and antigen tests, remain the commonly used tests [4,9]. While both these tests are highly specific, RT-PCR is more sensitive, making it a more widely used and acceptable test. Furthermore, radiologic assessments, including chest radiography or computed tomography (CT), were also used for the clinical diagnosis of COVID-19 infections [4,9,13,14].

Initial studies in China indicated fever, dry cough, difficulty in breathing, fatigue, myalgia, and low lymphocyte count and white blood cell count (some patients had normal white blood cell count) [15,16]. Most patients suffer from bilateral pneumonia; biomarkers also include increased IL-6, C-reactive protein (inflammation), neutrophil, and a decrease in lymphocytes. According to a study done with 138 patients in China, common symptoms were fever (98.6%), dry cough (59.4%), myalgia (34.8%), and dyspnea (31.2%) [15]. A much larger study done in China with 1099 COVID-19 patients hospitalised at 522 sites across China showed that the most common symptoms were fever (43.8% on admission and 88.7% during hospitalisation) and cough (67.8%) [9]. Apart from the respiratory symptoms, gastrointestinal (G.I.) symptoms were also detected among COVID-19 patients. In a study of 651 patients, 74 (11.4%) patients were found to have G.I. symptoms, including diarrhoea, vomiting, and nausea [17]. Evidence has shown that SARS-CoV-2 can bind to ACE2 in the gastrointestinal tract and transmit via faeces [18,19]. Similar to other aspects of COVID-19, a shift in symptoms has occurred between the initial phase of the pandemic and the recent ones. A high proportion (40–45%) of asymptomatic COVID-19 infections also pose a challenge for diagnostics [20]. A study done in the USA with a sample of 5700 patients showed that fever only accounted for 30.7% of the patients; this is strikingly different from the previous studies in China, although fever was still the most common symptom [21].

Risk factors

COVID-19 is one of the diseases which is generally not age, gender, or race-specific; however, comorbidities with other communicable and non-communicable diseases are considered risk factors for COVID-19. Despite not being gender-specific, studies have shown that men are more prone to get infected than women [4,22]. Furthermore, associated risk factors generally remain associated with mortality and severe morbidity than getting infected. People of any age get infected; however, middle-aged adults remain at risk of hospitalisation. Mortality is also high in elderly patients above 60 years of age, and the infection is severe in people with underlying diseases and obesity. Children have been reported to be less infected and do not seem to express severe symptoms when infected with SARS-CoV-2. Older age, pulmonary problems, especially asthma, fibrosis, heart diseases, diabetes and obesity, cancer and other blood disorders, chronic kidney, and liver diseases, and weakened immune system due to HIV and modern-day surgeries are some of the common causes to increase in the risk of severe COVID-19 symptoms.

In COVID-19, the increase in neutrophils and decrease in lymphocytes correlates with the severity of the disease [4]. According to a study done with 1099 patients in China, lymphopenia was present in 83.2% of the patients [9]. Most patients developed lymphopenia during hospitalisation, and deceased patients developed more severe lymphopenia over time while having higher white blood cells and neutrophil counts than survivors. Also, significantly higher D-dimer, blood urea, and creatinine levels among the deceased patients have been observed compared to the other patients [16]. As the rise in D-dimer is directly linked to clot formation, anticoagulation in severe COVID-19 cases is highly recommended to improve the outcome [23].

SARS-CoV-2 variant strains

As an RNA virus, SARS-CoV-2 has undergone several mutations in the past two years of the COVID-19 pandemic, leading to the development of different variants with changed genetic sequences compared with a reference sequence, Wuhan-Hu1. These substantial mutations are well backed up by the continued uncontrolled global transmission of this virus. Although most of these mutations are expected to be neutral, some of the new variants affect the transmissibility, infectivity, disease severity and even reduce vaccine efficiency [24,25]. So far, these mutations have resulted in five significant variants of concern – Alpha, Beta, Gamma, Delta, and Omicron. WHO has designated all these five variants as Variants of Concern (VOC) due to their increased transmissibility, increase in virulence, decrease in the effectiveness of available diagnostics, vaccines, and therapeutics [26,27]. The other variants, such as Eta, Iota, and Kappa, have not gained much interest and were never considered to be of any potential threat. All the mutations resulting in the formation of these variants ended up impacting the testing performances and vaccine efficacies. According to a recent meta-analysis, the overall vaccine efficacy among the fully vaccinated population for Alpha, Beta, Gamma, and Delta was 89%, 75%, 54%, and 74%, respectively [28]. Omicron has no such figures, owing to it being the youngest one among all these variants. Being the newest variant with the most mutations, all the involved organisations, including WHO and FDA, is paying the most attention to Omicron. Herein, we briefly describe all the five important variants of SARS-CoV-2:

Alpha – B0.1.1.7

The alpha variant was first identified in South-Eastern England in late September 2020 and rapidly gained global dominance in 192 different locations. CDC has categorised this variant as Variant Being Monitored (VBM) [29]. The higher transmissibility of this variant was related to higher prolonged viral shedding, an affinity for ACE2 receptor binding, fusogenicity and other sociodemographic variables

[30]. Despite being variations in different studies, the alpha variant was reported to be 50% more transmissible than the Wuhan strain. The variant has 17 mutations, including N501Y substitution in the receptor binding domain (RBD) region [31]. This variant was also associated with increasing infectivity and mortality, with an increase in R0 between 50% and 100% [32] and a hazard ratio of 1.55 (95%CI: 1.39–1.72) [33]. The vaccine efficacy of Pfizer dropped from 95% against the wildtype to 89% against the Alpha variant [34].

Beta – B0.1.351

The beta variant, first identified in South Africa in December 2020, was the variant responsible for the second COVID-19 wave in South Africa. This variant was categorised as a highly transmissible VOC, being identified at 139 locations worldwide. Despite being highly transmissible, by around 50% more than previous variants, the Beta variant was rarely associated with severe diseases. The variant has eight mutations in the spike protein, including three substitutions (K417N, E484K, and N501Y) in the RBD region, which resulted in immune escape and reinfection [35]. Also, a reduction in vaccine efficacy was detected; Novavax reduced from 89.3% to 49.4%, Johnson and Johnson reduced from 72% to 57%, AstraZeneca reduced from 75% to 10%, and Pfizer reduced from 95% to 75% [34,36]. The reduction in vaccine efficacy has been correlated to the K417N mutation, which was additional to that of the alpha variant.

Gamma – P.1

Gamma variant was first identified in Brazil in early January 2021 and was categorised as a VOC two times more transmissible than non-VOC; this variant has been verified from 98 location sites worldwide. The variant has ten mutations in the spike protein, including three key mutations (L18F, K417N, E484K) in the RBD region [37]. The Gamma variant has been shown to be resistant to monoclonal antibody neutralisation [38].

Delta – B0.1.617.2

Out of all the variants so far, the Delta variant was the most severe and was first identified in India in December 2020. This variant was responsible for the deadliest second COVID-19 wave in India. Being categorised as VOC and highly transmissible, this variant has been found in 176 places globally. This variant has several mutations apart from the ones found in previous variants. Apart from other mutations, the L452R and P681R mutations were associated with the increased infectivity of this variant. Compared to the Alpha variant, the Delta variant has 60% more transmissibility and was two times more severe to cause hospitalisation. Studies also report that previous infection cannot protect patients from the Delta variant. According to an epidemiological study conducted in India, the Delta variant escaped immunity in 34.6% of individuals who had a prior infection with SARS-CoV-2% and 57.0% higher infectivity than the wildtype [39,40]. The efficacy of the Pfizer vaccine has dropped from 93.7% against the Alpha variant to 88.0% against the Delta variant, and AstraZeneca has dropped from 74.5% against the Alpha variant to 67.0% against the Delta variant [41]. Although vaccines are less effective against the Delta variant, it was observed that vaccination protected infected patients from severe diseases and hospitalisation [41].

Omicron – B0.1.1.529

The most recent variant, Omicron, was first identified in South Africa in November 2021 [42]. This variant has been found globally in 77 different locations covering the six major continents within less than a month. It has the most mutations out of all the five variants, and some of these mutations, such as N501Y, D614G, K417N, T478K mutations and previously uncharacterised mutations, are concerning [43]. In a recent report by South African health insurer Discovery Health on December 14, 2021, the Omicron variant is more transmissible, more resistant to vaccines but causes less severe infections than the previously identified variants of SARS-CoV-2. According to GISIAD, within the first two months after the initial report of Omicron, over 80% of sequenced COVID-19 samples were verified as Omicron, which makes it the fastest growing VOC so far [44].

Several non-scientific reports claim the Omicron variant's ineffectiveness against all the current COVID-19 vaccines, whereas some agencies also claim that these vaccines prevent the hospitalisation of infected patients. According to a recent study, the sera induced by Omicron spike protein using mice shown to have weak neutralization in against the prototype, Alpha, and Delta pseudoviruses when compared to the wild-type (WT) spike protein; while the WT spike protein induced sera is 452 times weaker against the Omicron pseudoviruses [45]. Further analysis by He and colleagues has shown that both the WT spike protein and Omicron spike protein can induce Th1 and Th2 response; however, the WT spike protein has increased percentage of IFN-γ-secreting memory CD4 + and CD8 + T-cells, while the Omicron spike protein could only increase percentage of IFN-γ-secreting memory CD8 + T-cells when compared to the control group. The results indicated that immunization using Omicron spike protein could result in weak humoral and cellular immunity.

In recent development, more variants of Omicron have appeared, namely BA.1, BA.2, and BA.3 [46]. The Omicron BA.1 was the original Omicron variant discovered in November 2021 and it sequences as a monophyletic clade rooted within the B.1.1 lineage (Nextstrain clade 20B), with no clear basal progenitor [47]. More recently BA.2 and BA.3 have emerged, both sharing many mutations with BA.1, but also having unique mutations of their own. Although BA.2 and BA.3 are evolutionarily linked to BA.1, the three sub-lineages evolved independently from one another along separate branches [47]. Currently, Omicron XE and XL have also emerged, more scientific studies are needed for a better understanding of these two new variants [48,49].

Viral infection mechanism

Genetic similarities to SARS, beta-coronavirus, evolved from HKU9-1 (a bat coronavirus)

SARS-CoV-2 is a single-stranded, positive sense enveloped RNA beta-coronavirus in the same subgenus as the SARS-CoV [15]. The coronavirus has four structural component proteins, namely the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins [50] (Fig. 1). The S protein allows the virus to attach to the membrane of the host cell and the N protein holds the virus RNA genome, while E and M, along with the S protein, form a viral envelope [51]. The non-structural RNA genome of ORF1ab, ORF3, ORF6, 7a, 8, and ORF10 contains highly conserved information for genome replication [2,52]. According to genome sequencing, SARS-CoV-2 shares 79.6% similarity with SARS-CoV BJ01 [15]. Another study reported that around 77.2% of amino acids are identical to SARS-CoV [3]. SARS-CoV-2 shares a 50% similarity with MERS [53]. Further analysis indicates that SARS-CoV-2 and SARS-CoV belong to the same species and share the same ancestor. Both evolved from the bat coronavirus HKU9–1, and most of its phylogenetic inner joint neighbours and outgroups were found in various bats as natural hosts [54]. Therefore, it is very likely that bats are the natural host of SARS-CoV-2. The phylogenetic analysis of the full-length genome and RdRp and spike gene sequences (S) indicate that SARS-CoV-2 shares a 96.2% genome sequence with RaTG13, a bat coronavirus that can be found in Rhinolophus affinis from Yunnan province. A closer
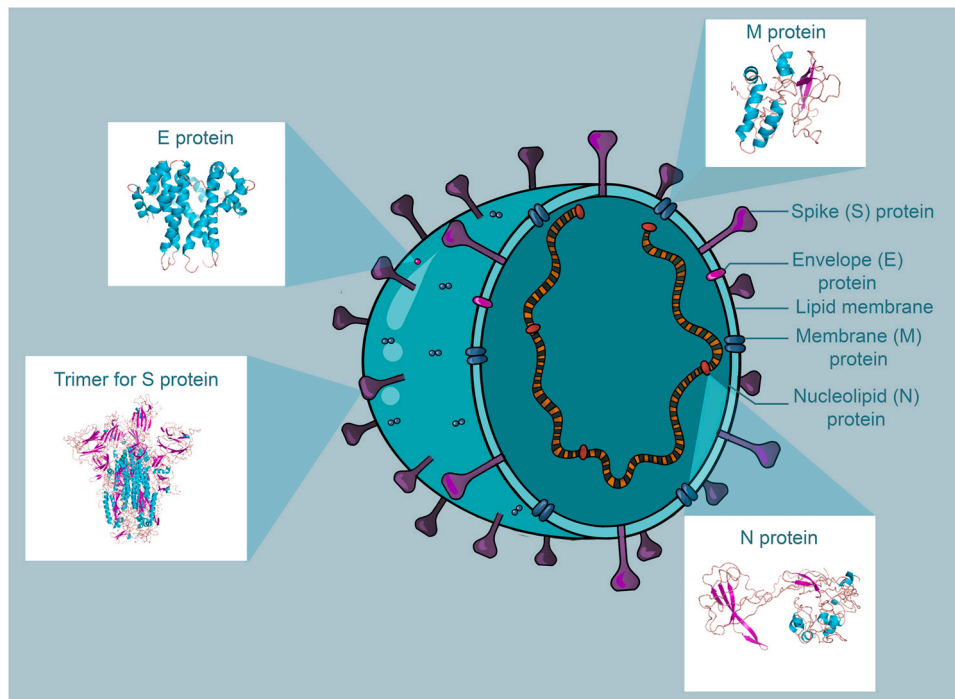
Fig. 1. Illustration of four important structural proteins of SARS-CoV-2.

look at the viral genome suggests that SARS-CoV-2 results from natural selection, and no evidence of recombination or human engineering is detected [2].

S1 spike protein

Coronaviruses use S protein for binding to host cell receptors. S protein is the primary antibody target and is of great interest in immunological responses and vaccine design. The S protein has two functional units – S1 and S2. S1 is responsible for recognising and binding to host cell receptors, while S2 is responsible for fusing with the host cell membrane [55–57]. SARS-CoV − 2 S protein is cleaved by a cellular protease called cathepsin L, thereby exposing the S2 domain of the S protein for membrane fusion, followed by endocytosis and forming low pH endosome [52].

The C-terminal RBD domain of the S1 protein is where the virus first contacts the host receptor [58]. The sequence of the S gene of SARS-CoV-2 showed a 75% nucleotide identity to SARS-CoV and 93.1% nucleotide identity to RaTG13. The major differences in the sequence of the S gene between SARS-CoV and SARS-CoV-2 are the three short insertions in the N-terminal domain and changes in four out of five of the key residues in the receptor-binding motif [15]. The Swiss model constructed a structural model of S protein to mock the binding to human ACE2 receptors, and the results showed kinetic energy of S protein binding for SARS-CoV-2 is at − 50.6 kcal/mol [54]. Although it is significantly lower than SARS-CoV at − 78.6 kcal/mol, it still has a strong binding affinity to human ACE2 protein. Although the RBD domain structure of SARS-CoV-2 has a high affinity of binding with human ACE2 protein receptor, it is not as optimal as the RDB domain of SARS-CoV, thus likely the result of natural selection [2]. In contrast, more recent studies have shown that SARS-CoV-2 has higher affinity of binding with human ACE2 protein receptor than SARS-CoV [59,60].

ACE2 protein binding

The RBD of SARS-CoV-2 S protein has shown a high affinity toward ACE2 protein receptors of humans, ferrets, cats, and other species with high receptor homology [2,15,61]. The ACE2 protein receptor has been the target of both SARS-CoV and SARS-CoV-2. ACE2 is a membrane-bound peptidase where the NH2-terminal peptide domain is the main component of the protein, with the catalytic site oriented extracellularly [62]. ACE2 is expressed in almost all the tissues, mainly in the ileum and kidney, followed by adipose tissue, heart, brain stem, lung, vasculature, stomach, liver, testis, and nasal and oral mucosa according to activity to data in the mouse that generally parallel ACE2 mRNA levels in humans [63–66]. ACE2 expression is higher in young people than in the elderly and higher among males than females [66–68].

Studies have indicated that the primary role of ACE2 is to convert AT-II into AT-1,7, controlling heart rate and reducing hypertension, vasoconstriction, sodium retention, oxidative stress, inflammation, and fibrosis, as well as enhancing baroreceptor sensitivity [69]. Consequently, the loss of ACE2, due to the destruction of the host cell by SARS-CoV-2, results in the halt of the AT-II conversion to AT-1,7 and causes the opposite effects, promoting an increase in blood pressure, inflammation, and fibrosis. This process may deteriorate as the immune response is activated and recruit's neutrophils to the infection site, resulting in ROS release and further increasing superoxide levels, thus inducing oxidative stress, complicating cardiovascular diseases. However, the use of ACE inhibitors to increase ACE2 activity has been shown in animal experiments; thus, it is not recommended for COVID-19 patients to discontinue RAS blockers [66]. This can be due to the destruction of ACE2 tissues by SARS-CoV-2 causes a shift in ACE1/ACE2 balance and using an ACE inhibitor to block ACE1 can archive homeostasis again [70].

Transmembrane protease serine 2 receptors

Studies have suggested that SARS-CoV-2 using transmembrane protease serine 2 (TMPRSS2) receptors to enter the host cell via ACE2 receptors by cleaving the S protein [71]. TMPRSS2 is widely expressed in epithelial tissues among human, including the lining of the upper airways, bronchi, and lungs [72]. The mechanism of coronavirus using TMPRSS2 cleavage to gain cathepsin L-independent entry and protection from IFITM proteins (an interferon) was already

discovered back in 2013 [73]. In 2019, an in vivo study has showed that TMPRSS2 knock out mice were significantly protected from inflammation caused by SARS and MERS infections [74].

The TMPRSS2 gene is greatly expressed in prostate tissues, and it has an important role in the development and progression of prostate cancer when fused with the v-ets avian erythroblastosis virus E26 oncogene homolog (ERG) gene; this results in a chromosomal rearrangement and mutation which disrupts prostate cellular downstream signals [75]. The possibility of men having higher prevalence of COVID-19 can be related to the over-expression of TMPRSS2 in men as compared to women [76]. Another study also found that even though there are no significant differences in TMPRSS2 expression between males and females in human lungs, males have a significant higher co-expression of TMPRSS2 and ACE2 pneumocytes I/II, which can be linked to higher prevalence and high mortality rates among male patients [77]. However, further studies in this area can unveil these corelations and related mechanisms.

Furin Protease

Furin protease is known to regulate viral entry into the cells and SARS-CoV-2 is no exception. Furin protease have been found to facilitate SARS-CoV-2 entry and viral fusion by cleaving the viral envelop glycoprotein [78]. This is rather unique since lineage B betacoronavirus, such as SARS-CoV and RaTG13, do not have furin-like cleavage site [79]. Insertion of furin-like cleavage site on the haemagglutinin receptor of influenza virus have shown to associate with increase in virulence [80]. A study has shown that addition of the furin-like cleavage site on the coronavirus spike protein receptor is associated with significant increase in fusion rate in cell-cell assay [81]. Whereas absence of the furin-like cleavage site has shown attenuation of SARS-CoV-2 [82].

The furin protease is highly expressed on epithelial cells within the mucosal membrane of oral cavity, which is also having high ACE2 expression [83]. The expression of furin protease is higher in tongue, gingiva, and lips, while low in the buccal and palatal tissues. These results indicate that the SARS-CoV-2 can efficiently gain entry via the oropharyngeal cavity route into both respiratory and digestive systems.

Sequenced strains and the mutation rate

The replication of the RNA virus genome is error-prone due to the lack of proofreading or post replicative RNA repair mechanisms [84]. Many mutations to the viral genes end up with no change or are detrimental to the virus [85]. This mutating property can often help the virus infect different species and adapt to a new environment [86].

Through the next-generation sequencing, the whole genome sequence of SARS-CoV-2 from patients in China in December 2019 indicated that the virus is very closely related to bat-SL-CoVZC45 and bat-SL-CoVZXC21, both are beta-coronavirus of bat origin, but the S gene has low identity with the two bar coronaviruses and is more closely related to SARS-CoV [53]. The genome of SARS-CoV-2 is about 79% identical to SARS-CoV and 50% identical to MERS-CoV. A typical RNA virus's average evolution rate is roughly 10–4 nucleotide substitutions per site per year, with mutation arising during every replication cycle [87].

According to a genomic analysis comparing ten different sequences of SARS-CoV-2 across the world (2 from China, two from the USA, one from Australia, one from South Korea, two from Taiwan, and two from Japan), no point mutation was found in E and M proteins, while the S protein had the most mutations [52]. Another study reported mutations occurring at positions 3036, 8782, 11083, 28144 and 26143. In contrast, lower mutation occurrences were found in

positions 1397, 2891, 14408, 17746, 17857, 18060, 23403, 28881, belonging to ORF1ab (1397 nsp2, 2891 nsp3, 14408 RdRp, 17746 and 17857 nsp143, 18060 nsp14), S (23403, spike protein) and ORF9a (28881, nucleocapsid phosphoprotein) sequences, respectively [88].

Geographical phylogenetic strain distribution

In a phylogenetic network analysis using 160 human coronavirus sequences, the strains were named A, B, and C, where A is the original strain of SARS-CoV-2 and the parent of B and B is the parent of C [89]. The network is a snapshot of the early-stage pandemic and found that A and C types are of significant proportion outside East Asia, while the B type is the most common in East Asia. The results possibly suggest that the B-type virus is immunologically or environmentally adapted to a large section of the East Asian population. The C type is the primary European type, while the A-type was found in American patients who had a resident history in Wuhan.

However, the methodology and interpretation of this analysis were criticised as the median-joining network is not a representation of viral evolution such as recombination and horizontal gene transfer [90]. Forster et al. responded, stating that they never claimed recombination had happened, and they used the median-joining network to display unresolved data conflicts as cycles (reticulations) [91].

The sequence of the SARS-CoV-2 isolated in Nepal is 29,811 nucleotides long, consists of 8903 (29.86%) adenosines, 5482 (18.39%) cytosines, 5852 (19.63%) guanines, and 9574 (32.12%) thymines [92]. BetaCoV/Nepal/61/2020 from coordinate 1–29811 is identical to the sequence of isolate 2019-nCoV WHU01 (GenBank accession number MN988668) from 15 to 29825, except with a replacement of a C from isolate 2019-nCoV WHU01 for a T at site 24019. While the sequence of BetaCoV/Nepal/61/2020 from coordinate 1–29811 is identical to the sequence of isolate Wuhan-Hu-1 (GenBank accession number NC_045512) from 16 to 29826, except with a replacement of a C from the isolate Wuhan-Hu-1 for a T at site 24019. The C24019T mutation corresponds to C24034T when using the sequence located under GISAID strain identifier EPI_ISL_405839 as a reference. This was a silent mutation at the S gene (codon AAC to AAT). Based on the reference sequence, five mutations were also identified: T8782C (in ORF1a, codons AGT to AGC, silent mutation), T9561C (in ORF1a, codons TTA to TCA, nonsilent mutation), C15607T (in ORF1b, codons CTA to TTA, silent mutation), C28144T (in ORF8b, codons TCA to TTA, nonsilent mutation), and T29095C (in nucleocapsid, codons TTT to TTC, silent mutation).

A phylogenetic analysis done in Italy with three complete genomes of 3 of the first 16 patients without foreign travel history has confirmed that the origin of SARS-CoV-2 strains in the study can be tracked several weeks before the first cases of COVID-19 pneumonia were described in China, and SARS-CoV-2 arrived in Northern Italy between the second half of January and early February 2020 [93]. The viral genomes isolated from these three patients have several different, mainly synonymous substitutions. The sequence of one patient living near the municipality where the highest number of cases were recorded showed a high degree of genomic heterogeneity, thus suggesting considerable genetic drift. The isolates reported from other European and Latin American patients indicated contact with Italy were closely related to the strain isolated during one of the first European clusters observed in Bavaria, Germany, in late January 2020. Another genomic analysis done in India of imported SARS-CoV-2 from Italy, Iran, and China has shown that the overall divergence of SARS-CoV-2 was about 99.97% [94]. The study collected 1920 nasal/throat swabs and obtained multiple sequence alignment of 21 complete genomes and 1563 full-genome sequences.
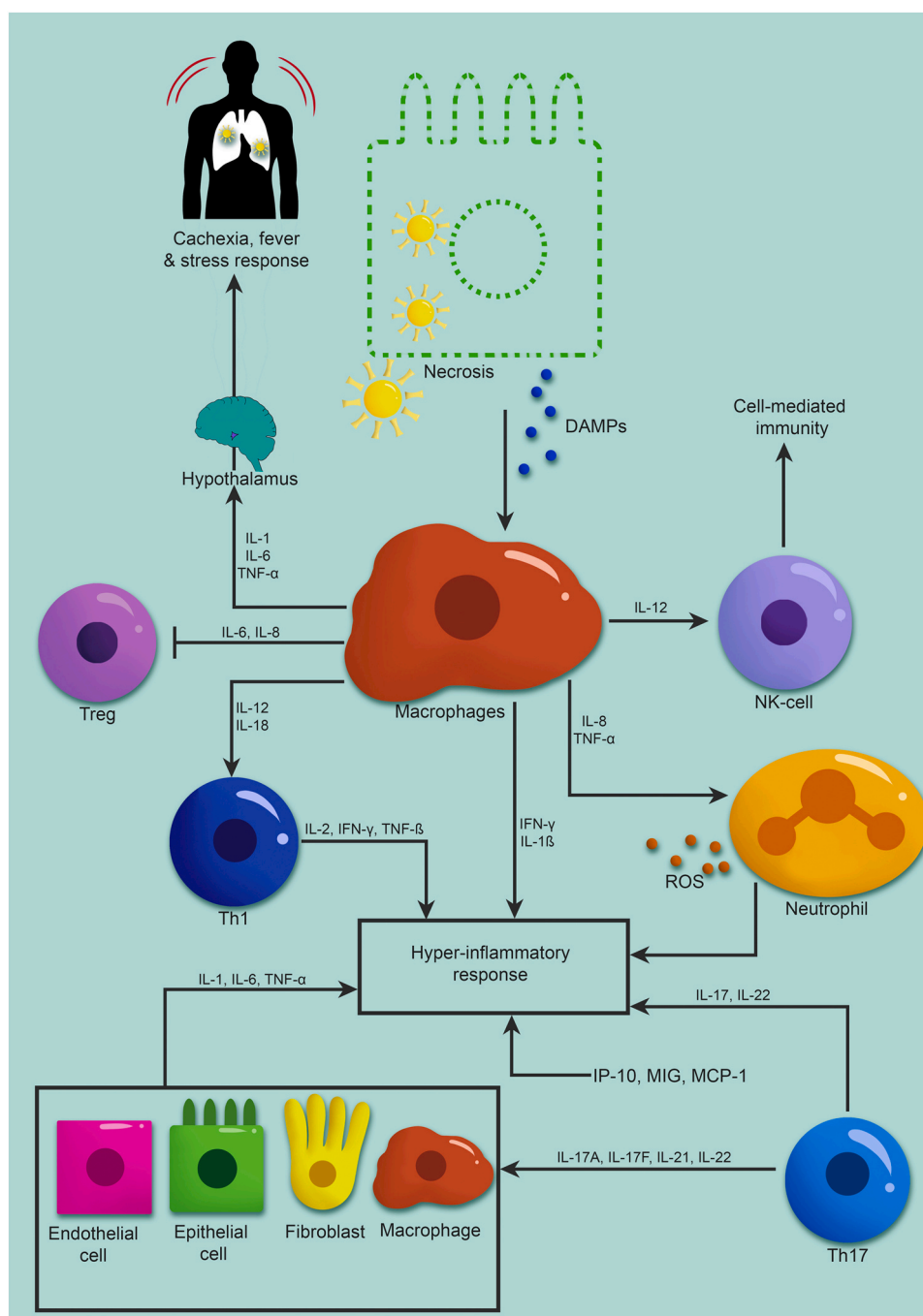
Fig. 2. : The immune response during the SARS-CoV-2 infection. Where the immune cells and cytokines activate to fight the viral infection, the virus infects an epithelial cell by attaching to the ACE2 receptor with help from the TMPRSS2 receptor. The natural killer (NK) cell recognises an infected cell and releases perforin and granzyme, causing apoptosis. Some viruses get phagocytosed by antigen-presenting cells and present the viral antigen (APC) on their surface. CD8 T-lymphocyte recognises the viral antigen presented on the infected cell surface or the APC, while CD4 + T-lymphocyte recognises the viral antigen presented on the APC and release cytokines causing chain reactions further downstream in the immune system.

Immune response

Defence mechanisms against coronaviruses

Being mainly a respiratory disease, SARS-CoV-2 affects the lungs in most patients; however, the initial site of infection is still unknown. A study of 41 patients showed an elevation of IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A, and TNFα in severe cases [95]. Lymphopenia and "cytokine storm" were also found common in critically ill patients [10] (Fig. 2). Death occurs due to multiple organ failures, especially among elderly patients. This may result from acute respiratory

distress syndrome (ARDS), septic shock, and a cytokine storm as the virus disseminates around the body and attacks ACE2 expressing cells. SARS-CoV-2 infection activates type 1 interferon (T1IFN) responses downstream with Th1/17 responses [10]. Efficient Th1 response was the main factor for the successful control of SARS-CoV and MERS-CoV and could likely be true for SARS-CoV-2 [10].

The innate immune cells need to recognise pathogen-associated molecular patterns (PAMPs) such as viral RNA via pattern recognition receptors (PRRs). SARS-CoV-2 has mechanisms that can inhibit the T1IFN response, such as decreasing STAT1 phosphorylation (Th1 subtype differentiation). Delaying T1IFN responses leads to an influx
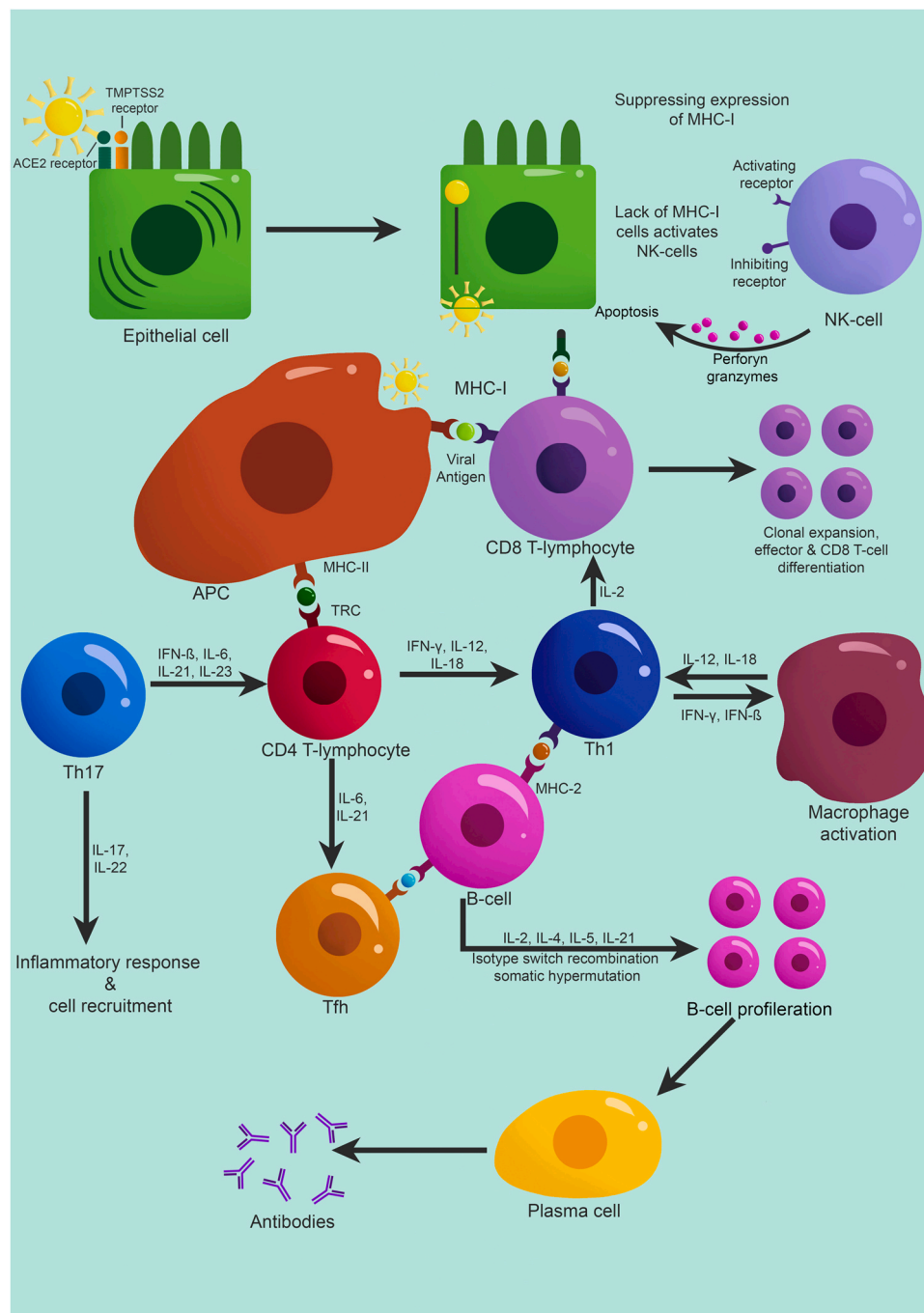
Fig. 3. : The immune response during a cytokine storm where the immune system causes damage to the body due to the SARS-CoV-2 infection. During SARS-CoV-2 infection, some infected cells die causing necrosis and releasing damage-associated molecular patterns (DAMPs). The macrophages react to the DAMPs and release cytokines which suppress regulatory T-cells (Treg) and activate other immune cells. This results in a chain reaction of hyper-inflammatory response and causes damage and stress to the body.

of hyper-inflammatory neutrophils and monocytes-macrophages (Fig. 3). Cytotoxic T lymphocytes destroy the virally infected cells. A study of one patient indicated that IgM peaked at day nine after disease onset and switched to IgG on week 2, and the study with an in vitro plaque assay showed that sera from all patients could neutralise SARS-CoV-2 [15].

Prompetchara et al. suggested that the T cell epitopes of the three coronaviruses can be studied for overlap similarities, which can help design a cross-reactive vaccine that protects against all three human coronaviruses in the future [10]. Currently, assumptions from SARS-CoV are used to estimate the immune responses for SARS-CoV-2. Observations suggest that coronaviruses are good at evading the host immune system. For example, MERS-CoV caused downregulation in MHC-I and MHC-2 when macrophages and dendritic cells were infected, and reduced T-lymphocyte activity [96]; and SARS-CoV has been observed to infect monocytes, macrophages, and T-lymphocyte [97]. The infection and manipulation of immune cells may explain the more extended incubation period of 2–11 days for SARS-CoV-2 as compared to 1–4 days for flu. SARS-CoV-2 relies on inhibiting the innate immune system and delaying the initial responses. The viral proteins, including membrane (M) or nonstructural (N.S.) proteins (e.g., NS4a, NS4b, NS15), are the key molecules in host immune modulation [10]. It is possible that these molecules can manipulate the microRNA mechanisms in the immune cells.

Leukocyte and lymphocyte count during infection

According to the initial study done in Wuhan, out of the 99 patients, 9% had leucocytes below the normal range and 24% above the normal range, 38% had neutrophils above the normal range, and 35% had decreased lymphocyte count, 4% had blood platelet decreased, and 12% had increased, and 51% had haemoglobin below the normal range [4]. Severe lymphopenia and a rise in white blood cell count have been associated with mortality. The reduction in lymphocytes may be due to SARS-CoV-2 infection of T-lymphocyte, while the rise of white blood cells, especially neutrophils, is due to the delayed T1IFN response while pro-inflammatory IL-6 and TNF-α expression responses are taking place [98,99]; where the virus already disseminated throughout the body and caused hyper-inflammatory response thus creates a cytokine storm.

Inflammation and inflammatory load

Over-secretion of inflammatory cytokines can be detected in severe COVID-19 patients. Studies in China have detected a rise in IL-1B, IL-1RA, IL-6, IL-7, IL-8, IL-9, IL-10, basic FGF, GCSF, GMCSF, IFN-γ, IFN-γ-induced protein (I.P.)− 10, monocyte chemotactic protein 1 (MCP1), MIP1A, MIP1B, C-reactive protein, and TNF-α in plasma concentrations among patients [15,95]. The severity of the disease in patients is associated with white blood cells, neutrophils, eosinophils, and lymphocyte counts. It is suggested that IL-2R level > 793.5 U/mL, WBC > 9.5 * 10^9/L, or neutrophil count > 7.305 * 10^9/ L indicate the progression of COVID-19 to critical conditions. These biomarkers indicate that inflammatory responses were correlated with the severity of SARS-CoV-2 [100]. Immunostaining of post-mortem tissue from patients who had died from COVID-19 has shown that macrophages express ACE2 and contain SARS-CoV-2 nucleoprotein, which also expresses IL-6. Expression of IL-6 further expresses macrophages with SARS-CoV-2 viral particles inside and is also associated with low lymphocyte count [101]. This is surprising as scRNA-seq analysis of human tissues does not find ACE2 in macrophages.

It is found that hyper-inflammation is characterised by a fulminant and fatal hyper-cytokinaemia with multi-organ failure resulting in secondary haemophagocytic lymphohistiocytosis and significantly contributes to severity and mortality [102]. However, immunosuppression is likely beneficial in such a scenario as data from a phase 3 randomised controlled trial of IL-1 blockade (anakinra) in sepsis showed significant survival benefits in patients with hyper-inflammation without increased adverse events [103]. It has been proven that the blockade of pro-inflammatory cytokine IL-6 is also beneficial for suppressing hyper-inflammation has been suggested as a therapy choice [101,104].

Tipping the scale- antiviral defence versus viral load

The immune cells will first detect viral PAMPs on the infected cell surface or viral RNAs via PRRs. The viral RNAs of SARS-CoV-2 can be detected by endosomal RNA PRRs such as Toll-like receptors (TLR-) 3 and 7 and/or cytoplasmic RNA sensors, namely retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) [99]. These receptors then activate downstream signal, and, in turn, triggers increased expression of T1IFN through IRF3 and other innate pro-inflammatory cytokines, including IL-1, IL-6, TNF-α through NFKB. T1IFN activate the IFN-α receptor complex and results in the phosphorylation/activation of STAT family transcription factors 1 and 2, which are essential for cellular antiviral response and activation of the adaptive immune system [105].

Although SARS-CoV-2 can suppress the immune response through alteration of ubiquitination and degradation of RNA sensors, the destruction of infected ACE2 expressing cells results in releasing

DAMPs and being detected by innate immune cell PRRs. The innate immune cells then release pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α, etc. This triggers adaptive immune cells to become involved in the defence against the viral infection. T lymphocyte is an essential component in the antiviral defence, where CD4 + T-cells differentiate and release cytokines to direct immune responses and activate B lymphocyte, CD8 + T-cells mediate destruction of infected cells and activate B-cells differentiate into plasma cells for antibody production.

Treatment modalities

The spectrum of medical therapies to treat COVID-19 is evolving rapidly, including both drugs approved by the U.S. Food and Drug Administration (FDA) and drugs made available under FDA Emergency Use Authorization (EUA). As shown in Fig. 4, several single-agent treatments and combinations are currently under investigation.

Plasma transfusion

At the early onset of the disease and due to the lack of any effective treatment available, support treatments were used to help patients fight back SARS-CoV-2 infection. Early observation of COVID-19 patients showed a rise of IgG and IgM in the blood, suggesting that collecting/manufacturing antibodies for passive immunotherapy may be effective in humans in neutralising the virus and preventing further infection. According to the study conducted by Zhou and co-workers, SARS-CoV-2 can be cross neutralised by using horse anti-SARS-CoV serum at a dilution of 1:40 [15]. However, later it was also reported that the use of sera of SARS patients has failed in cross-neutralising SARS-CoV-2 [2].

Drug repurposing

Drug repurposing, also known as repositioning, re-profiling, re-tasking, and rescue of drugs, is the process to identify new indications for existing drugs and is considered an efficient and economical approach to finding an effective drug to fight COVID-19 [106]. Several established medications, formerly discovered or used in the management of other diseases, are being tested for COVID-19 treatment, and some of them are being used to treat COVID-19 infection. Some of the examples of such drugs are discussed below:

Hydroxychloroquine and chloroquine

Hydroxychloroquine is an anti-malarial drug, and the use of hydroxychloroquine for COVID-19 has been debated at all levels.
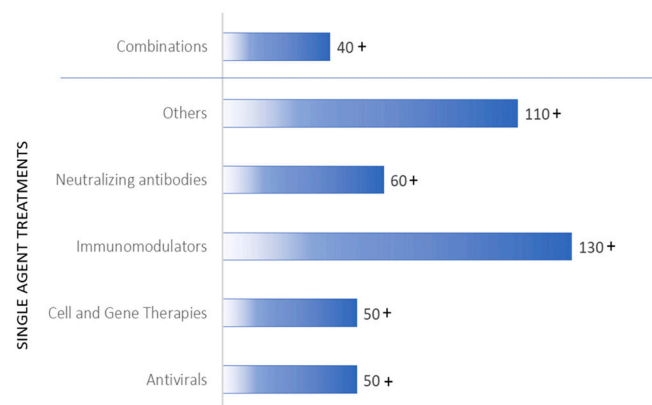

Fig. 4. : Type of COVID-19 treatment being studied. Corresponds to the number of safe to proceed INDs. Excludes INDs related to vaccines.

Hydroxychloroquine can disrupt the viral binding as SARS CoV requires certain acidic pH to fuse with host cells [107]. The weak base property of hydroxychloroquine can make the environment alkaline, which is assumed to work against SARS-CoV-2. However, this also causes side effects as the human body needs certain pH to achieve homeostasis. There are several clinical trials already looking at the effect of hydroxychloroquine against SARS-CoV-2 infection with very controversial outcomes. An uncontrolled study in France with 26 hospital inpatients has shown that 600 mg/day of hydroxychloroquine can reduce the viral shedding of SARS-CoV-2 and was more effective when combined with azithromycin [108]. However, another uncontrolled French study did not find hydroxychloroquine an effective drug for COVID-19 patients [109].

Another study was done to evaluate the effectiveness of hydroxychloroquine by giving a dose of 600 mg/day to ICU patients [110]. The study had 173 participants, including 84 in the treatment group and 89 in the control group. The results were not statistically significant as hydroxychloroquine did not affect SARS-CoV-2. Another study was conducted among patients with mild to moderate symptoms in China across 16 locations [111]. The study had 150 participants, including 75 in the treatment group and 75 in the control group. The results were not statistically significant and did not support that hydroxychloroquine is effective against SARS-CoV-2 infection.

Steroidal and non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are used to suppress the immune system to prevent collateral damage to patients, treating pain and inflammation. However, the side effects of NSAID long term use is associated with higher rates of cardiovascular outcomes such as myocardial infarction, heart failure, and stroke [112]. Evidence already showed that acute infection of SARS-CoV-2 puts stress on the respiratory and cardiovascular systems and increases the risk of stroke and myocardial infarction in patients. NSAIDs can further increase the risk of sepsis and cardiovascular or respiratory complications [113]. Observations in clinical settings have shown that cytokine storm is prevalent among severe patients where elevated pro-inflammatory cytokines spread around the body, similar to SARS and MERS [114]. NSAIDs have become an option to reduce the collateral damage of the cytokine storm. However, due to the side effects of NSAIDs and the nature of SARS-CoV-2 infection, balancing the risk and benefit ratio is a critical issue. The risks and benefits of using NSAIDs are still not clear and under intense debate. One of the main concerns is that suppressing the immune system can delay the elimination of viruses and secondary infections, especially in immunocompromised individuals. According to a report, severe patients usually undergo abrupt deterioration one to two weeks after the onset of symptoms. Anti-inflammatory therapy in this narrow time window is likely to achieve a favourable treatment response [114]. Several clinical trials were done for evaluating the effect of glucocorticoids on SARS as an immunomodulatory therapy, and the results of studies varied, and no consensus has been reached [115–119].

Corticosteroids such as dexamethasone have been used to treat very ill COVID-19 patients since the pandemic began. Patients who develop a hyper-immune response (a cytokine storm) to the viral infection have a high risk of damaging the lungs and other organs due to the immune system's overreaction, often leading to death. Dexamethasone and other corticosteroids (prednisone, methylprednisolone) are potent anti-inflammatory drugs. The NIH COVID-19 treatment guidelines recommend the use of dexamethasone based on the results of a trial in which 6000 patients hospitalised with COVID-19 randomly received either dexamethasone or standard treatment. Patients who required supplemental oxygen or ventilators and were given dexamethasone had a lower risk of dying within 28 days than those who received standard care. Dexamethasone had no effect on patients who did not require respiratory support [118].

The FDA has granted an emergency use authorisation for the rheumatoid arthritis drug baricitinib (Olumiant) to treat COVID-19 in some cases. Baricitinib is a pill that seems to work against COVID-19 by reducing inflammation and having antiviral activity. The FDA states baricitinib may be used in hospitalised people with COVID-19 who are on mechanical ventilators or need supplemental oxygen [120].

Antiviral drugs

Among different antiviral drugs, remdesivir was the most studied drug against SARS-CoV-2 infections. Remdesivir is an intravenous nucleotide prodrug of an adenosine analogue. Remdesivir binds to the viral RNA-dependent RNA polymerase and inhibits viral replication through premature termination of RNA transcription. Studies have shown that remdesivir demonstrated in vitro activity against SARS-CoV-2 [121]. Furthermore, in a rhesus macaque model of SARS-CoV-2 infection, the remdesivir-treated animals had lower virus levels in the lungs and less lung damage than the control untreated animals, suggesting in vivo efficacy of this drug against SARS-CoV-2 [122].

The FDA approves Remdesivir to treat COVID-19 in hospitalised adult and paediatric patients (aged ≥12 years and weighing ≥40 kg). It is also available through an FDA Emergency Use Authorization (EUA) for the treatment of COVID-19 in hospitalised paediatric patients weighing 3.5 kg to < 40 kg or aged < 12 years and weighing ≥ 3.5 kg [123]. Remdesivir should be administered in a hospital or a health care setting that can provide a similar level of care to an inpatient hospital.

In addition to remdesivir, other antiviral drugs that have been tested include favipiravir, ribavirin, darunavir, and combinations such as lopinavir and ritonavir have also been studied. Favipiravir (6-fluoro-3-hydroxy-2-pyrazinecarboxamide or T-705) is a nucleoside precursor which inhibits a broad range of influenza virus strains. Numerous studies have been undertaken to evaluate the efficacy of this drug against COVID-19, and a recent meta-analysis showed that favipiravir exerted no significant beneficial effect in terms of mortality in the general group of patients with mild to moderate COVID-19 [124]. Randomised trials were also conducted for this drug in combination with other drugs (Favipiravir + interferon-α, ChiCTR2000029600, favipiravir + baloxavir, ChiCTR2000029544) against coronaviruses [124].

Ribavirin, a broad-spectrum antiviral drug, is a guanosine analogue approved for treating hepatitis C virus in combination and respiratory syncytial virus as monotherapy. SARS-CoV2 has a very high EC50 of 109.50 μM and a Vero cells selectivity index of more than 3.65. Ribavirin at a dose rate of 500 mg given 2 – 3 times/day in combination with other drugs such as lopinavir/ritonavir or interferon (IFN)-α through intravenous route for not more than ten days made the SARS-CoV2 infected patients more resistant to respiratory distress syndrome as well as death [125]. Other combination studies include ribavirin + interferon-alpha, lopinavir/ritonavir + interferon-alpha and ribavirin + lopinavir/ritonavir + interferon-alpha in patients with mild to moderate COVID-19 are underway, and the combination, ribavirin + lopinavir/ritonavir has shown promising results. Another antiviral drug, darunavir, a second-generation non-peptide protease inhibitor effective against HIV-1, has a distinct chemical structure that enhances binding affinity and reduces dissociation rate, making it more potent than the other protease inhibitors [126]. Using computational drug design methods, darunavir was identified as one of the promising hits for inhibiting chymotrypsin-like protease of SARS-CoV2 [127]. In an in vitro study, darunavir at 300 μM concentration was found to inhibit SARS-CoV2 virus replication by 280 times in comparison to the untreated group, and it has been used (600 mg tablet every 12 h) along with other antiviral drugs and supportive therapy in the clinical management of COVID-19 patients in Italy [128]. Arbidol, also known as umifenovir, is a potent broad-spectrum antiviral agent, effective against numerous pathogenic respiratory viruses, and relatively safe for use

[129]. Arbidol and arbidol mesylate were reported to act directly on viral replication of SARS-CoV at an early stage in vitro [130]. Arbidol is one of the drugs in clinical trial phase 4 for pharmaceutical interventions of COVID-19, and treatment of COVID-19 patients with arbidol leads to a reduction in the mortality rate and an increase in the recovery rate [131,132].

Monoclonal antibodies

Monoclonal antibody medications that have been used against COVID-19 include sotrovimab and a combination of two antibodies called casirivimab and imdevimab. These drugs have been used to treat mild to moderate COVID-19 patients with a higher risk of developing severe illness due to COVID-19. Treatment consists of a single intravenous infusion given in an outpatient setting. These medications need to be given soon after COVID-19 symptoms start and prior to hospitalisation to be most effective. The FDA has also authorised the use of casirivimab and imdevimab as a treatment for people at higher risk of serious illness who have recently been exposed to the COVID-19 virus or are at high risk of exposure. A clinical trial was conducted using tocilizumab, an immunosuppressant that interrupts IL-6 release, on 21 severe COVID-19 patients [133]. The results have shown an immediate improvement in most patients' symptoms, hypoxaemia, and CT scan opacity changes. Bevacizumab, a monoclonal antibody that serves as a medication directed against the signalling protein VEGF (vascular endothelial growth factor) in various cancer treatments, is being evaluated for COVID-19 treatment. The drug suppresses tumours by inhibiting the growth of blood vessels that feed the tumour. By suppressing VEGF, this drug can also potentially reduce vascular permeability and decrease the amount of fluid entering the lungs of patients with COVID-19 suffering from ARDS. Tocilizumab and sarilumab, both monoclonal-antibody antagonists of the IL-6 receptor usually used to treat rheumatoid arthritis, are also being tested. A significant improvement in survival of patients with COVID-19 who were receiving tocilizumab, an interleukin (IL)−6 inhibitor, albeit with a very modest reduction of mortality (31% vs 35% with usual care, p = 0·0028), has been observed [134,135]. Other studies with tocilizumab and other IL-6 antagonists, such as sarilumab, have shown only minor, or no, reduction in mortality [136,137]. Casirivimab and imdevimab are the two neutralising mAb that work against the surface spike protein of SARS-CoV-2 and appear to maintain activity against variants of concern (VOC). This medication is only offered to patients ≥ 12 years at high risk for COVID-19 hospitalisation or complications. Sotrovimab is a single mab that has activity against VOCs. It has been granted emergency use authorisation (EUA) for outpatients with risk factors based on 1% vs 7% placebo hospitalisation or death (85% reduction) [138]. Casirivimab/imdevimab received EUA to prevent COVID-19 in patients at high risk after close contact (15 min cumulatively in a confirmed case over 24 h). Dosing is the same as treatment (600 mg/600 mg) but can be given S.Q. (requires four 2.5 mL injections) or IV infusion.

Antibiotics

The most frequently used antibiotics to treat SARS-CoV-2 patients for bacterial superinfections infections were fluoroquinolones, macrolides and cephalosporins [139]. A possible explanation for such use is lung coverage for pneumococcal, Gram-negative, and atypical bacterial infections. Ventilator-associated infections were reported in many studies without mentioning any specifically involved organism. It is unclear why and how antibiotics were used in most of these studies. Various studies have shown that most bacterial pneumonia diagnosed early in COVID-19 patients can be safely and effectively treated with broad-spectrum antibiotics [140–142]. A recent meta-analysis revealed that only 7.0% of hospitalised COVID-19 patients had a bacterial co-infection [143]. Another multi-centre study showed that only 86 out of 905 (9.5%) confirmed COVID-19 patients were clinically diagnosed with bacterial co-infection [144]. This implies that only a few COVID-19 patients would need antibiotics for possible bacterial pneumonia and other superimposed co-infections [145].

Over 80 clinical trials of azithromycin for COVID-19 are planned or underway [146], but only a few have reported results. Azithromycin has been evaluated as part of a hospital-based, open-label, randomised clinical trial of different COVID-19 treatments in the U.K. [147]. Recent findings show that azithromycin should not be used routinely to treat COVID-19 in the community in older adults without additional indications [148]. Four separate studies comparing survivors and non-survivors found no significant difference in antibiotic use [149–152]. However, there was no study on survival rates according to the presence or absence of empiric antibiotic treatment. Antibiotic efficacy should be further investigated prospectively in COVID-19 patients to minimise their irrelevant use. Using antibiotics to treat COVID-19 might encourage patients to believe that antibiotics are an appropriate treatment for other viral respiratory infections. Therefore, it is essential for clinicians to avoid prescribing antibiotics to patients seeking treatment for COVID-19 in the absence of an additional indication. Recent studies suggest that patients with altered gut microbiota might experience more severe COVID-19 symptoms [153]. Antibiotics may alter digestive microbial flora further, so empiric treatment for bacterial pneumonia in COVID-19 patients should only be initiated when clinical suspicion is high. The COVID-19 guidelines of the China National Health Commission (CNHC) [154] has stated that the blind or inappropriate use of antibiotic drugs should be avoided, especially broad-spectrum antibiotics.

Miscellaneous drugs

Some drugs used to treat different disease conditions with different mechanisms of action have also been studied for COVID19 treatment. The most promising ones undergoing clinical trials or have shown promise in initial studies are listed in Table 1.

Vaccines

Mass vaccination has already been agreed to be the most effective way to tackle the COVID-19 pandemic. Currently, there are three main types of COVID-19 vaccines that are authorised and recommended or undergoing large-scale (Phase 3) clinical trials in the United States. Each type of vaccine prompts our bodies to recognise and protect us from the virus that causes COVID-19. mRNA vaccines contain material from SARS-CoV-2 that signals human cells to make a viral receptor on the cell membrane surface. After the cells make the receptors, the genetic material from the vaccine gets destroyed. The immune system recognises the receptor antigen to develop memory T- and B-lymphocytes that engages SARS-CoV-2 in future infections. Protein subunit vaccines include antigens of the virus that are recognised by immune system as foreign protein, which then lead to the development of T-lymphocytes and antibodies to engage the virus in future infections. Vector vaccines use recombinant target viral gene contained inside a viral capsule (viral vector). Once the viral vector infects the human cells, the genetic material is released and instructs the cells to make COVID-19 viral proteins. This prompts the immune system to create memory T-lymphocytes and B-lymphocytes that engages the virus in future infections.

Table 1
Miscellaneous drugs used in different disease conditions being studied for their COVID-19 efficacy.

Drug	Mode of action/Type	Use	Effectiveness against Covid19
Nafamostat and Camostat	Serine protease inhibitors	Used against pancreatitis in humans	Undergoing phase 2 and phase 2/3 clinical trials in the USA and Japan
Famotidine	H2 receptor antagonist	Heartburn medication	Undergoing phase 3 randomized trial in the USA
Umifenovir	Broad-spectrum antiviral	Prophylaxis for influenza virus A and B	Preclinical studies
Nitazoxanide	Blocks maturation of viral nucleocapsid N protein	Anti-infective, antiviral	In clinical trials
Ivermectin	Lipophilic macrolide	Broad-spectrum anti-parasitic drug	Undergoing clinical trials in India and the USA
Fluvoxamine	Immunomodulator and antidepressant	Used to treat obsessive-compulsive disorder	Undergoing clinical trials in the USA
Baricitinib	JAK1/JAK2 inhibitor	Used to treat rheumatoid arthritis	FDA EUA approved for ages ≥ 2 yrs COVID-19 in hospitalized adults and pediatric patients requiring supplemental oxygen, invasive mechanical ventilation, or ECMO.
Teicoplanin (Teichomycin A)	Natural glycopeptide antibiotic	Used for the treatment of gram-positive infections	Preclinical studies
Fingolimod (FTY720)	Sphingosine-1-phosphate (S1P) receptor modulator	Used for the treatment of patients with relapsing multiple sclerosis	In Clinical trials
Thymosin α1	Immunomodulator	Used as an anticancer treatment	Undergoing clinical trials in China
Colchicine	Tubulin disruption	Prophylaxis and treatment of gout flares. Treatment of Familial Mediterranean Fever (FMF).	Randomized clinical trials are ongoing
Methylprednisolone	Immunosuppressive and anti-inflammatory agent	Used as an anti-inflammatory agent.	Randomized clinical trials are ongoing
Doxycycline	binds to metalloproteases	Antibiotic	In Phase III randomized clinical study in France
Ulinastatin	Broad-spectrum serine protease inhibitor	Used for the treatment of severe sepsis and mild to severe acute pancreatitis	Undergoing Phase II and Phase III clinical trials in the USA and India

Vaccines with EUA or FDA approval

All the approved vaccines need to undergo scientific processes to confirm their effectiveness, quality, and safety. Due to the abrupt emergence and wild spread of COVID-19, there was limited time to follow all the required guidelines. The first FDA approved COVID-19 vaccine under WHO Emergency Use Listing (EUL) was the Pfizer/BioNTech Comirnaty vaccine and has been available since December 31, 2020, for use in individuals 16 years of age and older. The SII/Covishield and AstraZeneca/AZD1222 vaccines (developed by AstraZeneca/Oxford and manufactured by the Serum Institute of India and S.K. Bio, respectively) were given EUL on February 16, 2021. The Janssen/Ad26. CoV2.S. COVID-19 vaccine, developed by Johnson & Johnson, was listed for EUL on March 12 2021. The Moderna COVID-19 vaccine (mRNA 1273) was listed for EUL on April 30, 2021, and the Sinopharm COVID-19 vaccine was listed for EUL on May 7, 2021. The Sinopharm vaccine is produced by Beijing Bio-Institute of Biological Products Co Ltd, a China National Biotec Group (CNBG) subsidiary. The Sinovac-CoronaVac was listed for EUL on 1 June 2021.

The FDA has granted Pfizer-BioNTech COVID-19 vaccine full approval under EUA, marketed as Comirnaty, to prevent COVID-19 disease in individuals 16 years of age and older. The vaccine is also available under EUA, including for individuals 12–15 years of age and the administration of the third dose in some categories of immunocompromised individuals. In the U.S., more than 170 million people have been fully vaccinated against COVID-19, and more than 92 millions of them received the Pfizer-BioNTech vaccine. Followed Comirnaty, the EUA for the Moderna vaccine was also approved shortly afterwards, and then the Johnson & Johnson one-shot vaccine was also granted EUA in February 2021. The list of all the COVID-19 approved vaccines and their types, the developing company and the country of origin are detailed in Table 2. However, the inequality in distribution of vaccines has hampered and may hamper the effectiveness of mass vaccine campaigns in future as well [155,156].

Vaccines in clinical/preclinical development

Following the WHO vaccine tracker there are currently 112 COVID-19 vaccines in clinical and 184 in preclinical development. These candidate vaccines use one or the other platform listed in Table 3 for development and are currently in different phases of clinical development, as shown in Fig. 5. Of all these vaccines candidates, most of them are in Phase II and Phase III of vaccine development (Fig. 5).

The unique feature of SARS-CoV-2 vaccine research and development is a diversity of vaccines with a range of technology platforms being evaluated, including nucleic acid (DNA and RNA), virus-like particle, peptide, viral vector (replicating and non-replicating), recombinant protein, live attenuated virus, an inactivated virus approach [157]. The most promising vaccine candidates with different antigenic modes and their trial phases and sponsors are detailed in Table 4.

Nucleic acid vaccines include DNA vaccines and messenger RNA (mRNA) vaccines. The DNA vaccine functions by inserting the DNA plasmid of the viral antigen into host cells and this generates cellular and humoral antigen-specific immunity, allowing the host to mount an immune response to the target disease [158,159]. DNA vaccines, however, do raise the concern of the plasmid integrating with host DNA and disrupting the usual transcription. mRNA vaccines also direct the production of antigens. However, unlike DNA vaccines, mRNA vaccines do not integrate into the host genome, thereby lowering the risk of mutations [160].

Viral vector vaccine uses the capsule of a virus with a recombinant genome inside, enabling intracellular antigen expression and inducing a robust cytotoxic T-cell response leading to the elimination of virus-infected cells [161]. The advantages of viral vector vaccines are high-efficiency gene transduction, highly specific delivery of genes to target cells, induction of robust immune responses and increased cellular immunity. However, integrating recombinant plasmids into the host genome could lead to cancer.

Table 2
Authorized/ Approved vaccines worldwide.

Name	Vaccine Type	Primary developers	Country of origin
Comirnaty (BNT162b2)	mRNA-based vaccine	Pfizer, BioNTech; Fosun Pharma	Multinational
Moderna COVID-19 Vaccine (mRNA-1273)	mRNA-based vaccine	Moderna, BARDA, NIAID	US
AstraZeneca (AZD1222); also known as Vaxzevria and Covishield	Adenovirus vaccine	BARDA, OWS	UK
Sputnik V	Recombinant adenovirus vaccine (rAd26 and rAd5)	Gamaleya Research Institute, Acellena Contract Drug Research and Development	Russia
Sputnik Light	Recombinant adenovirus vaccine (rAd26)	Gamaleya Research Institute, Acellena Contract Drug Research and Development	Russia
COVID-19 Vaccine Janssen (JNJ-78436735; Ad26. COV2. S)	Non-replicating viral vector	Janssen Vaccines (Johnson & Johnson)	The Netherlands, US
CoronaVac	Inactivated vaccine (formalin with alum adjuvant)	Sinovac	China
BBIBP-CorV	Inactivated vaccine	Beijing Institute of Biological Products; China National Pharmaceutical Group (Sinopharm)	China
EpiVacCorona	Peptide vaccine	Federal Budgetary Research Institution State Research Center of Virology and Biotechnology	Russia
Convidicea (PakVac, Ad5-nCoV)	Recombinant vaccine (adenovirus type 5 vector)	CanSino Biologics	China
Covaxin (BBV152)	Inactivated vaccine	Bharat Biotech, ICMR; Ocugen; ViroVax	India
WIBP-CorV	Inactivated vaccine	Wuhan Institute of Biological Products; China National Pharmaceutical Group (Sinopharm)	China
CoviVac	Inactivated vaccine	Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products	Russia
ZF2001 (ZIFIVAX)	Recombinant vaccine	Anhui Zhifei Longcom Biopharmaceutical, Institute of Microbiology of the Chinese Academy of Sciences	China, Uzbekistan
QazVac (QazCovid-in)	Inactivated vaccine	Research Institute for Biological Safety Problems	Kazakhstan
COVIran Barekat	Inactivated vaccine	Shifa Pharmed Industrial Group	Iran
Abdala (CIGB 66)	Protein subunit vaccine	Center for Genetic Engineering and Biotechnology	Cuba
Soberana 02	Conjugate vaccine	Finlay Institute of Vaccines; Pasteur Institute	Cuba, Iran
MVC-COV1901	Protein subunit vaccine	Medigen Vaccine Biologics Corp.; Dynavax	Taiwan

OWS: Operation Warp Speed is a collaboration of several US government departments including Health and Human Services (HHS) and subagencies, Defense, Agriculture, Energy and Veterans Affairs and the private sector.
ACTIV: Within OWS, the US National Institutes of Health (NIH) has partnered with more than 18 biopharmaceutical companies in an initiative called ACTIV. ACTIV aims to fast-track development of drug and vaccine candidates for COVID-19.
COVPN: The COVID-19 Prevention Trials Network (COVPN) combines clinical trial networks funded by the National Institute of Allergy and Infectious Diseases (NIAID): the HIV Vaccine Trials Network (HVTN), HIV Prevention Trials Network (HPTN), Infectious Diseases Clinical Research Consortium (IDCRC), and the AIDS Clinical Trials Group.
COVAX: The COVAX initiative, part of the World Health Organization's (WHO) Access to COVID-19 Tools (ACT) Accelerator, is being spearheaded by the Coalition for Epidemic Preparedness Innovations (CEPI); Gavi, the Vaccine Alliance; and WHO.
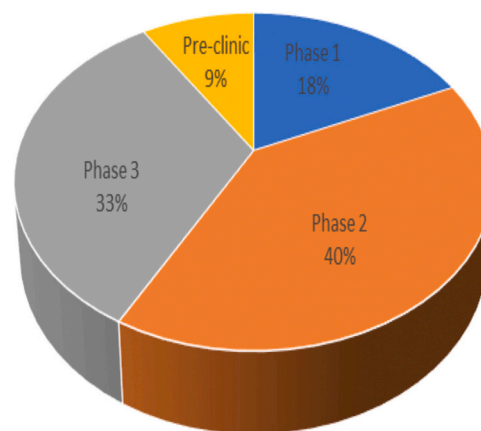
Table 3
Platforms used in the COVID19 vaccine development.

Platform		Candidate vaccines (No. and %)
PS	Protein subunit	38 (34%)
VVnr	Viral Vector (non-replicating)	17 (15%)
DNA	DNA	11 (10%)
IV	Inactivated Virus	16 (14%)
RNA	RNA	18 (16%)
VVr	Viral Vector (replicating)	2 (2%)
VLP	Virus Like Particle	5 (4%)
VVr + APC	VVr + Antigen Presenting Cell	2 (2%)
LAV	Live Attenuated Virus	2 (2%)
VVnr + APC	VVnr + Antigen Presenting Cell	1 (1%)

Fig. 5. : Percentage of vaccine candidates in different phases of development.

Convidicea (AD5-nCoV), Janssen (Ad26. COV2. S), Sinopharm (BBIBP-CorV), Covaxin (BBV152) and Sinovac (CoronaVac) have shown to cause reactions at the injection site, fatigue, headache, body aches and fever [162]. However, more serious adverse effects (though rare) such as allergies, myocarditis, pericarditis, and anaphylaxis have been found among people who received mRNA vaccines (Pfizer-BioNTech BNT162b2 and Moderna SpikeVax) [162,163].

Strength and limitation

The strength of this review is wide coverage of the COVID-19 pandemic across many different topics. The review gives the reader a basic understanding of the pandemic and the virus, its ability to mutate, spread rapidly, causing infection by evading host defence mechanisms and the immunological responses. The arduous and precipitous efforts to contain or stop the virus at the early onset of the disease with the help of support treatments to fight back SARS-CoV-2 infection, by repurposing existing drugs, using combination regimens, developing new drugs and vaccines have been discussed in this review. However, due to the wide coverage of the topic, some areas might not be covered deep enough. The other limitation is that some of the information may be outdated as COVID-19 is still evolving and changing.

Table 4
Vaccine candidates in development (pre-clinical or clinical phase). The most promising ones are listed.

mRNA based Vaccines		
Candidate	Trial Phase	Sponsor
ARCoV	Phase 3	Walvax Biotechnology Co., Ltd.; Abogen Biosciences Co. Ltd.; Yuxi Walvax Biotechnology Co., Ltd.
CVnCoV	Phase 2b/3	CureVac; GSK
BNT162	Phase 1/2/3	Pfizer, BioNTech
DS-5670a	Phase 1/2	Daiichi Sankyo Co., Ltd.
MRT5500	Phase 1/2	Sanofi, Translate Bio
PTX-COVID19-B	Phase 1	Providence Therapeutics; Canadian government
DNA Vaccines (Plasmid)		
ZyCoV-D	Phase 3	Zydus Cadila
INO-4800	Phase 2/3	Inovio Pharmaceuticals; Advaccine
COVID-eVax	Phase 1/2	Takis; Rottapharm Biotech
GLS-5310	Phase 1/2	GeneOne Life Science, Inc.
Covigenix VAX-001	Phase 1/2	Entos Pharmaceutics Inc.; Aegis Life, Inc.; Canadian Institutes of Health Research (CIHR), Canada
AG0301-COVID19	Phase 1/2	AnGes, Inc., Japan
GX-19 N	Phase 1/2	Genexine
CORVax12	Phase 1	OncoSec; Providence Cancer Institute
LineaDNA	Pre-clinical	Takis Biotech
Recombinant protein vaccine		
Vidprevtyn	Phase 3	Sanofi; GlaxoSmithKline
Nanocovax	Phase 3	Nanogen Biopharmaceutical, Vietnam
V-01	Phase 2	Guangdong Provincial Center for Disease Control and Prevention; Gaozhou Municipal Center for Disease Control and Prevention; Zhuhai Livzonumab Biotechnology Co., Ltd.
Razi Cov Pars	Phase 2	Razi Vaccine and Serum Research Institute
S-268019	Phase 1/2	Shionogi & Co., Ltd; Japan Agency for Medical Research and Development
202-CoV	Phase 1	Shanghai Zerun Biotechnology
Noora	Phase 1	Baqiyatallah University of Medical Sciences
NBP2001	Phase 1	SK Bioscience Co., Ltd.
PittCoVacc	Pre-clinical	UPMC/University of Pittsburgh School of Medicine
Protein subunit vaccine		
COVAC-2	Phase 1	University of Saskatchewan Vaccine and Infectious Disease Organization-International Vaccine Centre
UQ-CSL V451	Phase 1	CSL; The University of Queensland
AdimrSC-2 f	Phase 1	Adimmune
KBP-201	Phase 1	Kentucky BioProcessing, Inc.
Mambisa (CIGB 669)	Phase 1/2	Center for Genetic Engineering and Biotechnology
AKS-452	Phase 1/2	University Medical Center Groningen; Akston Biosciences
QazCoVac-P	Phase 1/2	Research Institute for Biological Safety Problems
SCB-2019	Phase 2/3	GlaxoSmithKline, Sanofi, Clover Biopharmaceuticals, Dynavax and Xiamen Innovax; CEPI
Adenovirus-based vaccine		
GRAd-COV2	Phase 2/3	ReiThera; Leukocare; Univercells
AdCLD-CoV19	Phase 1/2a	Cellid; LG Chem
ChAd-SARS-CoV-2-S	Pre-clinical	Washington University School of Medicine in St. Louis
Inactivated vaccine		
VLA2001	Phase 3	Valneva;UK National Institute for Health Research
KD-414	Phase 1/2	KM Biologics; Japan Agency for Medical Research and Development
FAKHRAVAC (MIVAC)	Phase 1	The Stem Cell Technology Research Center; Organization of Defensive Innovation and Research
Intranasal vaccine		
COVI-VAC	Phase 1	Codagenix; Serum Institute of India
MV-014–212	Phase 1	Meissa Vaccines, Inc.
BBV154	Phase 1	Bharat Biotech
DelNS1-nCoV-RBD LAIV	Phase 1/2	Xiamen University; University of Hong Kong; Beijing Wantai Biological Pharmacy; CEPI

#Data taken from RAPS (Regulatory Affairs Professionals Society) and WHO (World Health Organization).

Conclusion

Understanding the pathology, mechanisms of infection, and immunology of SARS-CoV-2 has been critical for developing therapeutic medications and successful vaccines against this pathogen that had catastrophic implications on all aspects of the life, throughout the globe. The ability of this virus to spread rapidly, undergo mutations, evade host defence mechanisms has been quite surprising for the scientific community and policy makers. This pandemic has exposed the lack of preparedness of policymakers and the scientific community for any sudden or uncertain pandemic that can happen anytime without admonition. The precipitous use of support medication, combination therapies, existing antiviral, antibiotic, antihistamine, anti-inflammatory and antimalarial drugs were used to contain or stop the deadly virus.

Since the virus was new and it spread so rapidly, preventive measure to contain the virus were the only option and later it was observed that this virus can evade host defence system and infect monocytes, macrophages, and T-lymphocytes among others. The infection and manipulation of immune cells later explained the more extended incubation period of 2–11 days for SARS-CoV-2 as compared to 1–4 days for flu. SARS-CoV-2 relies on inhibiting the innate immune system and delaying the initial responses. Being mainly a respiratory disease, it affects the lungs in most patients and death occurs due to multiple organ failures, especially among elderly patients. This may result from acute respiratory distress syndrome (ARDS), septic shock, and a cytokine storm as the virus disseminates around the body and attacks ACE2 expressing cells. The cytokine storm in COVID-19 patients was utilised to develop anti-cytokine therapies to treat SARS-CoV-2 infection. As the mutation of the RNA virus was rapid and unpredictable, the viral genome shift would have been detrimental to vaccine development. Furthermore, the assumption that previous SARS effective drugs such as hydroxychloroquine did not work with SARS-CoV2, complicated the use of known drugs to treat this pathogen and therefore new and specific drugs or strategies to treat or kill the virus were direly required.

Despite the magnitude of resources, funding, and global collaborations against COVID-19, it is obvious that more research is required to better understand this virus and prepare humanity for the eventuality of a pandemic in the future. Making a unified policy were all the stakeholders, policymakers, and scientific community work together and streamline their efforts would go a long way help contain and minimize the impact of any such pandemic that could happen in future. We anticipate that this work will go some way toward achieving that goal and will provide readers a clearer understanding of how to be more prepared for similar pandemics in the future.

CRediT authorship contribution statement

A.A., A.D., and A.K. conceived the idea; X.G., M.Y.W. and A.K. collected the data; A.K., M.Y.W. and A.A. analyzed the data; X.G., M.Y.W. and A.K. wrote the article; A.A. and A.D. edit and proofread the article. All authors approve the submission.

Conflict of interest

All authors declare no conflict of interest.

Ethics approval

Not applicable.

Data Availability

Not applicable.

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